HETEROLOGOUS EXPRESSION OF SECONDARY METABOLITE GENE CLUSTERS FROM ACTINOMYCETES

Juan Pablo Gomez-Escribano and Mervyn Bibb; Department of Molecular Microbiology, John Innes Centre, Norwich, NR4 7UH, United Kingdom; juan-pablo.gomez-escribano@jic.ac.uk

Key words: Streptomyces, heterologous host, genome mining

Introduction.
Actinomycetes are prolific producers of natural products, whose biosynthetic genes are usually grouped contiguously in the DNA forming gene clusters. The expression of a gene cluster from one organism in a different species is known as "heterologous expression," and it has been used with a range of purposes, from simply confirming the clustering of biosynthetic genes to producing variants of natural products by genetic engineering. Combined with comparative metabolic profiling, heterologous expression has become an important tool for the mining of the immense amount of genomic information obtained from very diverse actinomycete species, many of which are difficult to culture and manipulate genetically. In our laboratory we have developed protocols, genetic tools and host strains that we have used to clone and study the regulation and function of genes for the biosynthesis of diverse natural products.[1]

Methods. Gene clusters are isolated from *Escherichia coli* cosmid or PAC libraries. When necessary, the selected cosmid or PAC clones are made integrative by inserting chromosomal phage attachment sites by PCR-targeting.[2] These integrative constructs are transferred to the appropriate host strain typically by conjugation from *E. coli*. To study the function of a particular gene, its coding sequence is replaced by a resistance gene and, when necessary to avoid polar effects, the latter is removed to yield an in-frame deletion.[2,3] The resulting strains are then cultivated and analyzed for differential production of metabolites by comparative metabolite profiling.[1, 4]

Results. We recently engineered *Streptomyces coelicolor* strains for enhanced heterologous expression while at the same time greatly simplifying their extracellular metabolite profile.[5] These strains have been used for the production of a wide range of secondary metabolites. Taking advantage of the increased production levels delivered by our host strains, we have identified the biosynthetic gene clusters of tunicamycin[6], cypemycin[8], grisemycin[9], and are currently elucidating the biosynthesis and regulation of tunicamycin[7] and planosporicin[10]. These projects, and the strategies used to overcome the limitations of heterologous expression, will be discussed.

Conclusions. Heterologous expression facilitates the identification and study of the genes involved in the biosynthesis of natural products, the elucidation of the biosynthetic pathway and the regulation of gene expression.

Acknowledgements. Research funded by the UK Biotechnology and Biological Sciences Research Council (BBSRC) and by the European Commission.

References.

1. Gomez-Escribano J. P., Bibb M. (2012). *Streptomyces coelicolor* as an expression host for heterologous gene clusters. *Methods Enzymol*. 517:279–300.
2. Gust, B., Chandra, G., Jakimowicz, D., Yuqing, T., Bruton, C. J., & Chater, K. F. (2004). Lambda red-mediated genetic manipulation of antibiotic-producing *Streptomyces*. *Adv Appl Microbiol.* 54:107–128.
3. Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., & Hopwood, D. A. (2000). Practical *Streptomyces* genetics. John Innes Centre, Norwich Research Park, Norwich, England.
4. Zerikly M, Challis GL. (2009). Strategies for the discovery of new natural products by genome mining. *Chembiochem*. 10:625-33.
5. Gomez-Escribano J. P., Bibb M. J. (2011). Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. *Microb. Biotechnol*. 4:207-215.
6. Wyszynski F. J., Hesketh A. R., Bibb M. J., and Davis B. J. (2010) Dissecting tunicamycin biosynthesis by genome mining: cloning and heterologous expression of a minimal gene cluster. *Chem. Sci*. 1:581-589
7. Wyszynski F. J., Lee S. S., Yabe T., Wang H., Gomez-Escribano J. P., Bibb M. J., Lee S. J., Davies G. J., Davis B. G. (2012) Biosynthesis of the tunicamycin antibiotics proceeds via unique exo-glycal intermediates. Nat. Chem. 4:539-46
8. Claesen J, Bibb M. (2010) Genome mining and genetic analysis of cypemycin biosynthesis reveal an unusual class of posttranslationally modified peptides. *Proc Natl Acad Sci U S A*. 107:16297-302.
9. Claesen J, Bibb MJ. (2011) Biosynthesis and regulation of grisemycin, a new member of the linaridin family of ribosomally synthesized peptides produced by *Streptomyces griseus* IFO 13350. *J Bacteriol*. 193:2510-6.